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THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8865

APR 2 8 1975

Application for Research Grant
(Use extra pages as needed)

Date: April 15, 1975

1. Principal Investigator (give title and degrees):

Herbert McKennis, Jr., Ph.D.
Professor of Pharmacology

2. Institution & address:

Medical College of Virginia
Richmond, Virginia 23293

3. Department(s) where research will be done or collaboration provided:

Department of Pharmacology
(Division of Biochemical Pharmacology)
Medical College of Virginia, Richmond, Virginia 23298

4. Short title of study:

Transport and Metabolism of Amine Constituents of Cigarette Smoke

5. Proposed starting date: 1 June 1975

6. Estimated time to complete: 3 years

7. Brief description of specific research aims:

Various investigations of cigarette smoke have provided evidence for several hundred amine constituents which are absorbed, excreted, and metabolized to varying degrees by man and other animals. Common sense alone would serve to suggest that most of these substances are excreted in one form or another -- as opposed to being permanently and completely stored. Recent reports (Russell and Feyerabend, Lancet, 179 (1975); Falkman et al., Analyst, 100, 99 (1975)), emphasize more and more that significant detectable quantities of nitrogen bases from cigarette smoke are determinable by physical and chemical means in the blood of non-smokers as well as the blood of smokers. The nitrogen bases in cigarette smoke can be considered to range from the simplest ammonia to bases with more complex structure such as harmane and norharmane. Since claims have been made that many of the nitrogenous bases are directly or indirectly involved in the development of pathological conditions in man and animals, factors that determine the disposition of these nitrogen bases become increasingly important. Although pH effects on the urinary excretion of nicotine and a few related compounds have been reexamined in recent years by Beckett et al., various factors such as protein binding, renal tubular excretion and absorption, competition and renal metabolic changes, which are important to the elimination of the nitrogen bases are not well understood or even crudely defined for many important nitrogen bases that are derived directly or indirectly from tobacco smoke. A

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Number 7 continued:

list of some of specific substances where substantial data is required would include cotinine, cotinine-N-oxide, 4-(3-pyridyl)-4-methylamino-butyric acid, demethylcotinine, cotinine methonium ion, allohydrocotinine, 3-pyridylacetic acid and many secondary and tertiary amines which are capable of forming N-nitroso compounds and amine oxides directly or indirectly.

8. Brief statement of working hypothesis:

The rate of elimination of nitrogen bases assimilated as a result of tobacco use is dependent upon a variety of factors. These factors include metabolic destruction, excretory routes (renal, respiratory, etc.) and various competitive factors between the parent bases, their metabolites, and structurally related compounds arising from the diet or normal metabolism. It becomes important to determine these relationships since both parent bases and their real or hypothetical metabolites (for example, amine oxides and nitroso compounds) have been implicated in various disease processes. Once the processes and relationships are better understood, one can more easily determine whether or not existing and future health-related claims are sound or unsound. It should also then be easier to treat and study variation between individuals in terms of biological mechanisms and not merely as statistically different phenomena.

9. Details of experimental design and procedures (append extra pages as necessary)

The literature attributes to or implicates nicotine and related pyridine bases a role in production of beneficial effects and detrimental effects. Real or alleged detrimental effects include the enhancement of platelet aggregation that has been attributed to nicotine that is obtained from smoking (Levine, Circ. 48, 619 (1973)) and a possible relationship to carcinogeneses, which includes nitrosation of nornicotine in vivo ("Environment and Cancer"; pp 113-141, 1971) and an abnormal excretion pattern of nicotine metabolites (cotinine and nicotine-N'-oxide) related to bladder-cancer patients (Gorrod, Jenner, Keyzell, and Nikhael, J. Nat. Canc. Inst. 52, 1974). Beneficial effects include consolidation of learning as induced by cotinine and 3-pyridyacetic acid (Essman, 4th Inst. Cong. Pharm.(1969)), and through use of nicotine, nornicotine, and cotinine, a lowering in aggressivity problems (U.S. Pat. No. 3,870,794, Hutchinson et al.). Other possible beneficial effects include an inhibition in lipolysis brought about by 3-pyridylacetic acid and its glycine conjugatate. Comparative studies on biological properties these two nicotine metabolites have recently been described (Bowman et al. IRCS, 3, 65 (1975)). Experimental design and procedures are largely illustrated with the pyridine bases. The general design can however be applied to other nitrogen bases of smoke.

1. Preparation of Compounds: Both isotopic and non-isotopic synthesis can be conducted by procedures based on those previously described ("Tobacco Alkaloids and Related Compounds", U.S. von Euler ed., 1965, p 53 et seq. "Disposition and Fate of Nicotine in Animals" by McKennis). Recent modifications to the original methods have also been described in publications from this laboratory.

Number 9 continued:

2. Control of Urinary Elimination of Pyridine Bases: Investigations of Isaac and Rand (Eur. J. Pharmac., 8 269 (1969), Langone et al. (Arch. Biochem. Biophys. 164, 536 (1974), Haines et al. (Chem. Pharm. 16 1083 (1974), Falkman et al (Analyst, 100, 99, (1975), and others, including numerous unpublished observations, point to a rapid disappearance of nicotine from the blood of smokers. For many days after smoking, cotinine, allohydroxycotinine and other nicotine metabolites are excreted. The quantity of cotinine appearing in the urine, using the figures of Langone et al. (Biochem. 12, 5025, 1973), appears from all data, as calculated by us, to exceed the amount of cotinine available in the blood.

Since cotinine is a lingering compound and assumed by some to be carcinogenic (Boyland, Planta Med. Suppl. 13 (1968) it is desirable to determine why elimination of cotinine is slow and the nature of the cotinine reservoir in man and animals which is providing a source of urine cotinine beyond that seen from blood levels.

A. Possible Chemical Precursors of Cotinine

Animal data suggest that nicotine alone is not the source of the slowly eliminated and long-lingering cotinine. Blood data and previous radioautographic data (Bowman et al., J. Pharm. Exp. Therap., 143, 301 (1964)) Schmiterlow and Hanson in "Tobacco Alkaloids and Related Compounds", von Euler, ed. p. 75) emphasize the need to look for other sources.

We will, therefore, look for sources of cotinine other than nicotine for cotinine in the urine. Concurrently, one needs to know renal mechanisms for elimination of cotinine and determine whether or not protein binding is an important contributor to the slow release of cotinine. Proper candidates for the extra cotinine include 4-(3-pyridyl)-4-methylaminobutyric acid (McKennis et al., J. Am. Chem. Soc. 79 6342 (1957), cotinine-N-oxide, and nornicotine. To determine the feasibility of such studies, pilot studies have already been conducted in this laboratory with cotinine-N-oxide. Cotinine-N-oxide was determined chromatographically in processed urine and cotinine was isolated as a picric acid salt.

Although preliminary data indicates that cotinine-N-oxide may feed the cotinine reservoir, there is no data on the possible effect of the N-oxide of allohydroxycotinine on the reservoir. The synthesis of the N-oxide of allohydroxycotinine for the contemplated studies will involve the use of the intermediate dibromoticonine (McKennis et al., J. Chem. Soc. 2046 (1973)).

B. Renal Mechanisms

Despite continued focus on the multiplicity of metabolites and continuing need for knowledge on rate determining and competive processes, there has been relatively little said in the literature on the renal mechanisms for elimination of nicotine and its metabolites, aside from the modern investigation of pH effects that were originally put forth by Larson and coworkers many years ago and broadened by the recent studies of Beckett et al. The fact that additional data on renal mechanisms is required becomes very obvious from existing reports of Beckett et al., showing virtually no pH effects on various nicotine metabolites and demonstrable effects only indicated with nicotine (as earlier described by Larson et al.). The methods for the required studies are almost classical in many cases.

Number 9 continued:

Clearance of some of the quaternary ammonium nicotine metabolites can be compared with the established patterns for the normal dietary constituent trigonelline and N'-methylnicotinamide as described by Beyer et al (Am. J. Physiol. 160 311 (1950)). Both stop-flow free-flow methods can be employed. The amphoteric nature of many nicotine metabolites suggests that both the base transport system and the organic anion transport system will be involved. These notions can be tested by use of Cyamine 863, a potent inhibitor of the base transport system, and with Probenecid, an inhibitor of the organic anion transport system.

Preliminary feasibility studies have been started here on one quaternary ammonium ion metabolite, cotinine methonium ion. The results from this, where clearance of inulin was simultaneously measured, indicate that there is probably an active secretary mechanism for the metabolite and that protein binding is not an important factor. It may be imagined however that protein binding - not yet studied - is a highly significant factor in the slow elimination of cotinine from man and other species. Experimental data on this one point alone as generated in this project should do much to clarify claims that continue to arise.

3. Special Aspects of N-nitrosamine Formation In Vivo: It is apparent that because test results indicate that approximately 85% of the N-nitrosamines thus far tested show carcinogenic activity that all of these substances will be suspect for many years. Leaving aside the reported occurrence of N-nitroso compounds in smoke and tobacco itself, the facilitation of N-nitrosamine formation in vivo can be considered to take place if secondary or tertiary amines (Smith, The Chemistry of Open-Chain Organic Nitrogen Compounds, vol. 1 (1965)) react with nitrite that is obtained from the diet. It is often considered that the stomach is an advantageous spot for such a reaction. For instance, the fact that nicotine is secreted into the stomach makes nicotine a proper candidate, and the hypothetical product would be N-nitrosonornicotine. Easier to see is the reaction to form N-nitroso compounds from the two metanicotine isomers (Sprouse et al., Coresta Abstracts 32, (1972)) and from dihydrometanicotine. These two or three metabolites of nicotine have been studied, in part, in this laboratory, but cis-metanicotine metabolism has been much neglected. The competive nature of biotransformation of these compounds will be studied with the aid of ^{14}C labelled material. Previous informal reports to the Council for Tobacco Research - U.S.A. have indicated that the oxidative series of metabolic reactions for elimination of these three bases is initiated by diamine oxidase and probably inhibited by histamine (competitive) and possibly also inhibited by some the commonly employed antihistamines.

10 Space and facilities available (when elsewhere than item 2 indicates, state location):

Two laboratories (approximate total of 800 sq. ft.), well-equipped for chemical and pharmacological studies, are available for these studies. In addition, there are two instrument rooms which house spectrographic, chromatographic, and radioactive counting equipment. Animal quarters (shared with others) are available for small mammals and large animals (horses, etc.) are kept in rented areas or at a school animal farm.

List of some major items of permanent equipment available for this work:

Cary recording spectrophotometer, model 11-PM
Grass polygraph, six channel, model 5
Nuclear-Chicago liquid scintillation system, 720 series
Beckman aminoacid analyzer, model 120B
Perkin-Elmer gas chromatograph, model 801
Nuclear-Chicago gas chromatography counting system
Wilken Aerograph Autoprep, model A-700
Preiser Scientific integrator-printer
Wilkens Aerograph 200 (2 each)
Nuclear-Chicago Actigraph III paper radio chromatography system
International preparative ultra centrifuge, model B-35
Vacuum pumps (six of various types)
Warburg Apparatus
Hewlett Packard Model 5700A Gas Chromatograph with integrator
Chemical balances (4 each)
Zeiss photoelectric polarimeter
Cahn electrobalance
Fraction collectors (2 each)
Miscellaneous glass metabolism cages, distillation equipment, chromatography equipment
Radiometer pH meter, O_2CO_2 determinator
Blood oxygenator (local design for organ perfusion)
Varian A-60 NMR apparatus
DuPont Model 830 Liquid Chromatography Apparatus
Varian M-66 Mass Spectrometer
Packard Tri-Carb Tissue Oxidizer
Bechman LS-50 Liquid Scintillation System
Aminco-Bowman Spectrophotofluorometer
Chronolog Platelet Aggregometer with Recorder

Additional medium resolution mass spectrometer service is available with data handling through State Laboratories.

11. Additional facilities required:

If any, these would be determined by the outcome of the investigation.

12. Biographical sketches of investigator(s) and other professional personnel (append).

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

Number 13:

Five Most Recent and Pertinent Publications of Investigators:

1. Bowman, F. J. and Foulkes, E. C., Antidiuretic Hormone and Urea Permeability of Collecting Duets, Am. J. Physiol. 218, 231 (1970).

2. Bowman, F. J., Bowman, E. R., and McKennis, H., Jr., Effects of 3-Pyridylacetic Acid in Rabbit Epididymal Fat Pads, IRCS 3, 65 (1975).

3. McKennis, H., Jr., Bowman, E. R., Quin, L. D., and Denny, R. C., J. Chem. Soc. Perkin Trans. I, 2046 (1973).

4. Bowman, E. R., Chang, R. S. L., Sprouse, C. T. and McKennis, H., Jr., N-3-Pyridylacetylglycine as a Nicotine Metabolite, Abstracts of Papers, 27th Tobacco Chemists' Res. Conf., 1973 p 32.

5. McKennis, H., Jr., Chang, R. S. L., Bowman, E. R. and Wilson, K. L., Jr., Effects of Isomers of Metanicotine on Smooth Muscle, Fed. Proc., 33; 470 (1974).

14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
Edward R. Bowman, Ph.D.	50%	11,000
Herbert McKennis, Jr., Ph.D.	10%	no request
Faye J. Bowman, Ph.D. (not related to E. R. Bowman)	10%	no request
Technical		
Kendall Wilson M.S.	100%	10,752
Graduate Student (not selected)	ca 50%	3,700
Graduate Student (not selected)	ca 50%	no request
Sub-Total for A		25,452
B. Consumable supplies (by major categories)		
Animals and animal care		3,500
Chemicals and glassware		1,900
Isotopes		1,750
High Speed liquid chromatography columns		450
Sub-Total for B		7,600
C. Other expenses (itemize)		
Communication		250
Travel (Scientific paper presentation and Conferences on project related material)		625
Mass spectrometer service		1,200
Sub-Total for C		2,075
Running Total of A + B + C		35,127
D. Permanent equipment (itemize)		
N/A		
Sub-Total for D		---
E. Indirect costs (15% of A+B+C)		5,269
Total request		40,396

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	31,000	8,500	3,000	3,000	6,375	51,875
Year 3	34,000	8,500	3,000	---	6,825	49,325

16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Gifts	Annonymous and American Tobacco Co.	$33,000	June 30, 1974 July 1, 1975

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Cardiovascular Effect of Nicotine Metabolites	N.I.H.	101,879	May 1, 1975 June 30, 1978

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Herbert McKennis, Jr.

Signature Herbert McKennis, Jr. Date 15 Apr 1975

Telephone 804 (Area Code) 770-4406 (Number) N/A (Extension)

Checks payable to

Walter Lossing, Comptroller

Mailing address for checks

Medical College of Virginia

1200 East Broad Street

Richmond, Virginia 23298

Responsible officer of institution

Typed Name Martha Bell Conway, Administrator

Title Research Grants and Contracts

Signature Martha Bell Conway Date 4/24/75

Telephone (804) (Area Code) 770-4443 (Number) (Extension)

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Dr. Herbert McKennis, Jr.
Medical College of Virginia
Richmond, Virginia

Born: REDACTED
Citizen

Education:

Harvard S. B. 1938 (field of concentration - chemistry).

Polytechnic Institute of Brooklyn 1939 - 1942 - graduate student in chemistry.

Cornell University Ph.D. 1945 (major subject - biochemistry; minor subjects - physiology and pharmacology).

Experience:

1955 - present	Professor of Pharmacology Medical College of Virginia
1960 -	Visiting Professor Institute of Physiology, University of Chile
1953 - 1955	Associate Professor of Research Pharmacology Medical College of Virginia
1949 - 1953	Head, Basic Sciences-Research Department Naval C. E. Research and Evaluation Laboratory Port Hueneme, California (and Solomons, Maryland)
1948 - 1949	Associate Professor of Biochemistry Department of Surgery Medical College of Virginia
1946 - 1948	Instructor in Physiological Chemistry School of Medicine, The Johns Hopkins University
1945 - 1946	Assistant Professor of Chemistry Medical College of Virginia
1942 - 1945	Assistant in Biochemistry Cornell University Medical College
1940 - 1942	Chemist Ciba Pharmaceutical Products, Summit, New Jersey
1938 - 1939	Chemist Nuodex Products Company, Elizabeth, New Jersey

Honorary Member:

REDACTED

Professional Societies:

REDACTED

REDACTED

REDACTED

Edward R. Bowman
Medical College of Virginia
Richmond, Virginia

Born: REDACTED
Citizen

Education:

Concord College B.S. 1952 (Biology - Chemistry).

West Virginia University 1953 (Physiology).

Duke University 1955-56 (Graduate Student in Physiology).

Medical College of Virginia Ph.D. 1963 (Pharmacology).

Experience:

1961 - present	Research Associate Department of Pharmacology Medical College of Virginia Richmond, Virginia
1958 - 1961	Graduate Student, Major - Pharmacology Minor - Physiology & Biochemistry Medical College of Virginia Richmond, Virginia
1956 - 1958	Research Assistant Department of Pharmacology Medical College of Virginia Richmond, Virginia
1955 - 1956	Graduate Student, Major - Physiology Minor - Anatomy Duke University Durham, North Carolina
1954 - 1955	Bacteriologist State Department of Health Richmond, Virginia
1952 - 1953	Graduate Student, Physiology West Virginia University Morgantown, West Virginia
1952	Student, Biology & Chemistry Concord College Athens, West Virginia
1950 - 1951	U.S. Army
1947 - 1950	Student, Biology & Chemistry Concord College Athens, West Virginia
1944 - 1946	U.S. Army

Professional Societies:

REDACTED

REDACTED

Curriculum Vitae

1. Personal Information:

1.1 Alta Faye Bowman (Maiden Name: Johnson)
1.2
1.3
1.4 REDACTED
1.5
1.6 REDACTED

1.7 Medical College of Virginia
Department of Pharmacology
Room 434, McGuire Hall Annex
Richmond, Virginia 23298
(703) 770-4683

2. Licensure:

2.1 N/A
2.2 N/A

3. Education:

Vanderbilt University
Nashville, Tennessee Ph.D. 1967

Tennessee Polytechnic Institute
Cookeville, Tennessee M.A: 1962
B.S. 1961

4. Military Service Record:

None

5. Postdoctoral Training, or Special Experience:

None

6. Academic Appointments or Other Significant Work Experience:

Research Associate
Department of Pharmacology
Medical College of Virginia
Richmond, Virginia 1970-Present

Research Assistant
Kettering Laboratory
University of Cincinnati
Cincinnati, Ohio 1967-1969

7. Membership - Scientific, Honorary and Professional Societies:

REDACTED

CURRICULUM VITAE

NAME: Kendall Louis Wilson, Jr.

PERMANENT ADDRESS:

DATE OF BIRTH: REDACTED

PLACE OF BIRTH:

MARITAL STATUS: REDACTED

SECONDARY EDUCATION: Marion High School
Marion, Maryland

HIGHER EDUCATION:

Dates	Institution	Degree
1966-1970	Randolph-Macon College Ashland, Virginia	B.S. in Biology
1970-1974	School of Graduate Studies Medical College of Virginia Richmond, Virginia	M.S. in Pathology

Thesis Title: "An Investigation of Momordica balsamina as an Antifertility Agent"

POSITIONS HELD:

1967-1968	Laboratory Assistant - General Biology Randolph-Macon College, Ashland Virginia
1969	Laboratory Instructor - Histology Randolph-Macon College, Ashland, Virginia
1970-1973	Laboratory Specialist, Clinical Pathology (Hematology and Clinical Microscopy Labs) Medical College of Virginia Richmond, Virginia
1973-Present	Research Assistant - Department of Pharmacology Division of Biochemical Pharmacology Medical College of Virginia Richmond, Virginia

1973-Present Laboratory Specialist, Night Service Clinic
Medical College of Virginia
Richmond, Virginia (10 hrs/week)

HONORS:

MEMBERSHIP: REDACTED